Spatial and temporal patterns of occurrence of *Lutzomyia* sand fly species in an endemic area for cutaneous leishmaniasis in the Atlantic Forest region of northeast Brazil

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ABSTRACT: Sand fly populations of different ecological niches in the Amaraji endemic American Cutaneous Leishmaniasis (ACL) focus of the Pernambuco Atlantic Forest region of northeastern Brazil were monitored spatiotemporally. *Lutzomyia whitmani* was dominant in all niches but occurred in smaller numbers in forested locations. *L. whitmani* was significantly less seasonal than the other species, being present throughout the year while other species were more abundant between February and April. These results suggest that *L. whitmani* may potentially be the principal vector of ACL in the region, even though the sand fly fauna was diverse: 88% were *L.whitmani* and 12% belonged to 11 other species. Two other species, *L. complexa* (1.3%) and *L. migonei* (0.8%), considered to be ACL vectors in other regions, were also present. This detailed picture of the sand fly population's abundance and spatiotemporal distribution provides a basis for future modeling studies of forecasting sand fly activity patterns and ACL occurence. ***Journal of Vector Ecology* 36 (Supplement 1): S71-S76. 2011.**

Keyword Index: American cutaneous leishmaniasis, *Lutzomyia whitmani*, Atlantic forest, spatiotemporal distribution.

INTRODUCTION

In the New World, American cutaneous leishmaniasis (ACL) is a zoonotic disease caused by different species of *Leishmania* and it is one of the most important health problems in Brazil, where some 35,000 cases occur each year (Secretaria de Vigilância em Saude, Ministerio da Saude, 2010). In humans, infections may be inapparent, but in others cases the clinical spectrum ranges from localized, often self-healing cutaneous lesions (CLs) to severe and mutilating mucocutaneous lesions (MCL) or diffuse CLs (Grimaldi and Tesh 1993, Carvalho et al. 1994). A particularly high prevalence of ACL has been reported in recent years in Pernambuco state, northeastern Brazil, in the vicinity of remnant patches of Atlantic forest (Brandão-Filho et al. 1999). The rapid increase in cases, coupled with evidence of peridomestic transmission at a number of sites (Campbell-Lendrum et al. 2001), has prompted calls for preventative measures to be added to the national control policy of diagnosis and treatment.

Phlebotomine sand fly species have been incriminated in transmission of ACL in the Americas (Marcondes 2001, Shaw 2002), and both epidemiological and experimental evidence indicate some degree of vector-parasite specificity. The enzootic cycle depends on close sand fly-reservoir contact and is considered a major factor limiting the geographic distribution of the disease. Although finding known vector species in new regions is interesting, detailed studies are required to determine their vectorial importance. An important step in this process is a better understanding of their distribution in different ecological niches during the different seasons of the year.

Lutzomyia whitmani is one of the most important vectors of *Leishmania* (*Viannia*) *braziliensis* in Brazil (Rangel and Lainson 2009) and has been collected in large numbers in the Atlantic Forest region of Pernambuco (in Portuguese *Zona da Mata*), in the municipality of Amaraji. It has also been found infected naturally with *L.* (*V.*) *braziliensis* in this region and the parasites belong to a zymodeme found in both humans and mammalian reservoir hosts in the same area. These observations add considerable weight to conclusions regarding its importance as a vector of zoonotic ACL cycle in this region (Brandão-Filho et al. 2003, Brito et al. 2009).

The aim of the present study is to provide additional data on the ecology of sand flies in the Amaraji region. We summarize one year's detailed sampling of sand flies in different ecological niches of a highly endemic ACL region in the Atlantic Forest zone of eastern Pernambuco. Unlike similar studies in other foci, our sampling was performed simultaneously at different sites and repeated throughout the year, allowing evaluation of occurrence patterns in both dimensions simultaneously. Only via such intensive sampling is it possible to understand their activity and consequently their vector potential. The present paper is the first major step in the analysis of this extensive data set.

MATERIALS AND METHODS

Sand flies were captured using ten CDC light traps with incandescent light sources set in different ecological niches during 4 successive days each month. The study was

carried out in the localities of Refrigerio and Tranquilidade, in the Amaraji municipality (8°22'59"S 35°27'09"W, 289 m), Pernambuco state (Figure 1). Sampling occurred in nine sessions during 2009 represented by a total of 256 trap-nights. A village or ranch was visited and sampled every second month, which permitted large numbers of sites to be sampled and different types of sites visited in alternating pairs of months. In this area, small patches of rainforest are surrounded by extensive areas of sugar cane and banana plantations, which are the dominant cash crops for the local people. Traps were positioned in places previously chosen, corresponding to sites in remnant forest patches, in plantations, and around houses (peridomicile). Sites for traps were recorded in the field using a hand-held Garmin (eTrex® HC series model) global positioning system, accurate to ~10 m on the ground. Field samples were stored separately by trap and night, permitting detailed tracking of the spatial dimensions of the occurrence of each individual sand fly captured.

Once field sampling was completed each month, each sand fly captured was identified to species. These data were summarized in various manners, visualizing patterns of occurrence of each species through time and space.

We tested for differences in seasonality of occurrences between the most common species and the next-less-common species by the following rarefaction procedure. For the less-common species, we calculated the proportion of positive trap nights for each month, and calculated the standard deviation of these monthly proportions through the year. For the more-common species, of the positive trap-nights, we sub-sampled 100 times positive trap-nights at random in numbers matching the number of positive trap-nights for the less-common species, and calculated the standard deviation of the monthly proportions through the year for each of the 100 replicates. Finally, we compared the observed value for the less-common species with the distribution of rarefied replicate values for the more-common species to establish a one-tailed probability distribution for the comparison. We linked seasonality of sand fly occurrences qualitatively to seasonal climatic variation by comparison with regional climate interpolations at 0.17° spatial resolution (Hijmans et al. 2005).

RESULTS

Table 1 summarizes captures of the various *Lutzomyia* species over the study region and through the study period. *L. whitmani* was the dominant species, with 1,191 individuals, constituting 88.0% of the total of sand flies captured. This overwhelming abundance was particularly intense near rural buildings and domiciles, with 93% of the total; in forested situations, *L. whitmani* still dominated, but with only 42% of the total. The dominance of this species was almost total near rural buildings, as the next-most-common species, *L. evandroi*, constituted only 5.0% of the total captures, and the remaining ten species only accounted for 7.0% of the total. Other species, such as *L. evandroi*, *L. quinquefer*, and *L. migonei,* were found in small numbers at sites near rural buildings occasionally. In forested sites, *L. whitmani* continued to be the dominant species, but here *L. evandroi*, *L. tupynambai*, and *L. complexa* were taken. Athough the sand fly fauna of the Amaraji region is diverse, with 12 species detected, it is nonetheless quite monospecific functionally. The 21 trap-nights in which sampling was unsuccessful represented 8.3% of the total set of trapping nights.

Seasonal patterns of occurrence contrasted between *L. whitmani* and the remaining species. *L. whitmani* was found in every sampling period, in the forest and near anthropogenic sites, with the greatest numbers being taken in March. However, the numbers of this species were also moderate to high in samples from June-October (i.e., >35 individuals in each monthly sample). In contrast, occurrences of the remaining species were highly concentrated in the period between February and April; only 41 individuals of any species other than *L. whitmani* were captured after April. The rarefaction-based comparisons of captures of *L. whitmani* and *L. evandroi* (the second-most-common species) indicated that *L. whitmani* is significantly less seasonal than *L. evandroi*. This period of concentrated occurrence from February to April coincided with the initiation of the yearly rainy season, although occurrences dropped off by May and June, which are the actual peak months of rain.

The spatial distribution of sand fly captures was also highly heterogeneous and variable (Figure 2). For example, *L. whitmani* occurred more frequently in the northeastern-most sites as well as in a few scattered localities of four other northern sites. However, none of the southwestern sites yielded specimens of this species and it was also absent in several other sites, including two sets close to the northeastern-most sites. *L. evandroi* was also taken frequently in the northeastern sites and was found at only one other site across the entire region. The remaining species showed a pattern similar to that of *L. evandroi*, being present in several northeastern sites and in only two other sites in the entire sample set.

DISCUSSION

Many Brazilian species of *Lutzomyia* have been associated with ACL transmission. In this study, we identified 12 species, some of which have been previously incriminated as competent leishmaniasis vectors. This diverse sand fly community is nonetheless dominated by the known vector species *L. whitmani*.

Our sampling at Amaraji shows that *L. whitmani* is the dominant presence throughout the year, particularly at sites near rural houses and other human facilities. Species diversity of sand flies was higher at forested sites than near human buildings, where *L. whitmani* was so dominant. *L. whitmani* has been perhaps the species most frequently connected to ACL transmission in the west-central, northern, and northeastern parts of Brazil. It has been captured in Brazil in many endemic ACL areas, in different vegetation and climate contexts (Costa et al. 2007,

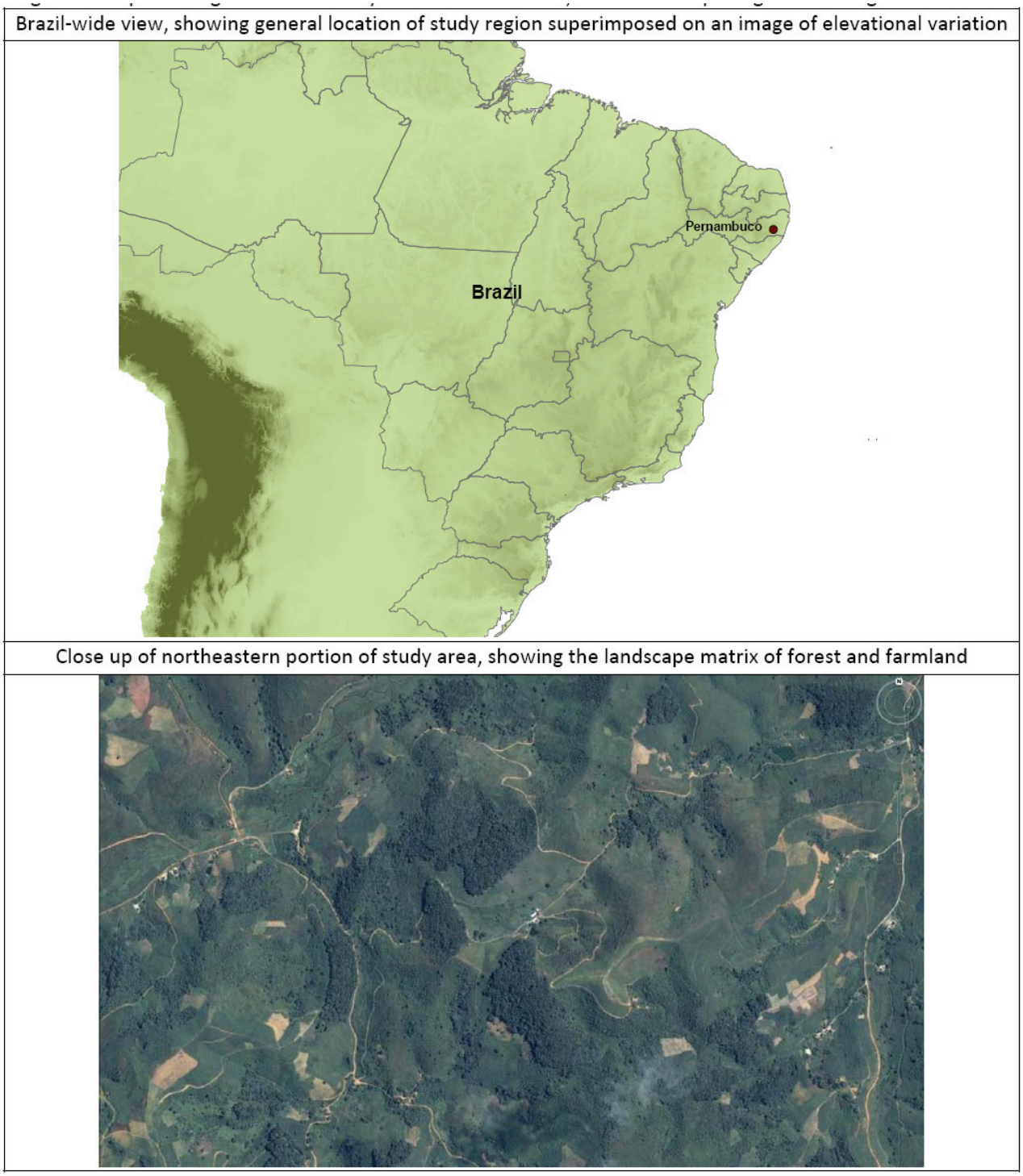

Figure 1. Map showing location of study area in Pernambuco, Brazil. Close up image from Google Earth.

Zeilhofer et al. 2008, Souza et al. 2002, Peterson and Shaw 2003, Queiroz et al. 1994). More recently, several studies have indicated the increasing adaptation of *L. whitmani* to peridomiciliary sites: this trend would seem to be a positive response to human encroachment linked to deforestation (Costa et al. 2007, Campbell-Lendrum et al. 2000, Brandão-Filho et al. 2003, Souza et al. 2002, Teodoro et al. 1999). Another species, *L. migonei,* is also known to be involved in peridomiciliary ACL transmission in many regions of Brazil (Queiroz et al. 1994, Mayo et al. 1998, Azevedo and Rangel 1991), although it was uncommon in our samples.

L. evandroi and *L. quinquefer* have been captured near domestic animal shelters and peridomiciliary situations, but they have never been incriminated as vectors for human leishmaniasis. (Ximenes et al. 1999, Souza et al. 2002). Others species captured in the Amaraji region, such as *L. fischeri*, *L. longispina*, and *L. sordellii* (Souza et al. 2002, Luz et al. 2000, Mayo et al. 1998), have been encountered

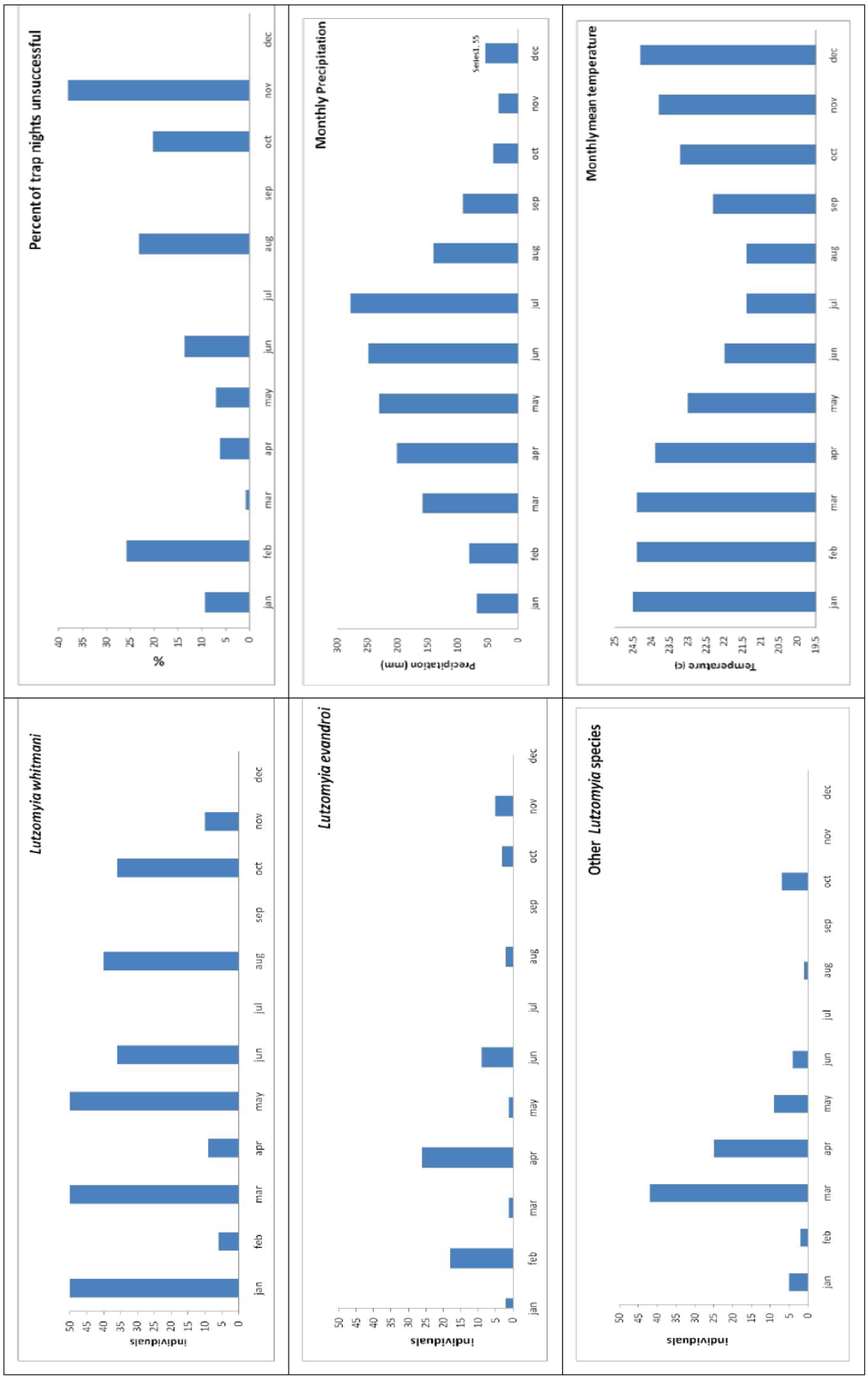

Figure 2. Monthly trends in trap success (in terms of number of individuals for *Lutzomyia whitmani*, *L. evandroi*, and other species; also shown are percentage of trap-nights yielding no captures ("negatives"), and yearly trends in mean annual temperature and annual precipitation. Note that occurrences of *L. whitmani* in January, March, and May exceed the maximum visible in the chart, with 186, 716, and 152 individuals captured, respectively.

in peridomicilary habitats and forested environments in cutaneous and visceral leishmaniasis endemic areas.

Simultaneous monthly sand fly captures in different ecological niches throughout the year enabled us to evaluate species diversity, seasonality, and abundances at different sites in different environments. As in other sand fly studies in the Brazilian cerrado and forested environments (Costa et al. 2007, Campbell-Lendrun et al. 2000, Luz et al. 2000), we found minimal seasonality of *L. whitmani* in Amaraji, although it was taken in greater numbers between March and June, when precipitation is increasing in the region. Increasing the frequency of sampling could result in clearer definition of peaks of abundance and better association with climatic events. Determining seasonal trends of sand fly populations is difficult since high variability characterizes nightly capture rates. *L. whitmani* was also seen to be most associated with peridomiciliary environments, emphasizing its potential vectorial importance to humans within these habitats. Curiously, in Mato Grosso do Sul, no marked seasonality was observed in this species, and it was present in larger numbers in forested habitats rather than in anthropogenic sites (Galati et al. 1996).

Seasonality was most evident in samples from forested sites, with captures of all species concentrated in March through May at the beginning of the rainy season. Besides *L. whitmani*, other common species included *L. evandoi*, *L. tupynambai*, and *L. complexa*. The latter species has been identified as an important vector associated with autochthonous ACL transmission in Para state, in the Amazon region, and other Atlantic Forest sites in Pernambuco (Souza et al. 1996, Andrade et al. 2005, Carvalho et al. 2007). Our results nonetheless reinforce the role of *L. whitmani* as the dominant vector of ACL transmission in this region, as even in samples at forested sites, it was the dominant species.

L. whitmani populations have been studied and characterized in terms of host and site preferences, dispersal, genetic variation, and natural infection rates (Campbell-Lendrum et al. 1999a,b). *L. whitmani* were significantly more attracted to humans than to dogs or chickens, showing very anthropophilic behavior. Experimental comparisons of anthropophily between geographically separated populations of this species have been developed (Campbell-Lendrum et al. 1999b, 2000). Comparing levels of anthropophily between sand fly species and populations of the same species from different geographical regions is complicated due to variations in their relative density. With these limitations in mind it would seem that the North-South and Amazonian mitochondrial lineages of *L. whitmani* are more anthropophilic than the North-Eastern lineage. Intraspecific comparisons among non-Amazonian sites suggest that *L. whitmani* is less anthropophilic than *L. intermedia* but more so than *L. longipalpis* (Brazil et al. 1991, Campbell-Lendrum et al. 1999a).

The combination of present and past studies on the biology of *L. whitmani* reinforce its status as the principal vector of ACL in peridomicillary and degraded forest habitats in the Atlantic rainforest zone of Pernambuco state. Such data sets will also enable us to make spatial associations of sand fly distributions with ACL cases, characterization of sand fly ecological niches, and forecasting of seasonal spatial activity patterns.

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